Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 10, 2015

Original Article

ANTI-DIABETIC EFFECT OF POLYHERBAL FORMULATION IN OGTT AND STREPTOZOTOCIN-INDUCED DIABETIC RAT MODEL

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Received: 28 Jun 2015 Revised and Accepted: 08 Aug 2015

ABSTRACT

Objective: The present study investigates the efficacy of DiaKure, a poly herbal formulation composed of powder of different herbs on blood glucose level of normal and diabetic rats.

Methods: The raw materials were collected made it into a powder formulation. Streptozotocin 60 mg/kg was administered as a single i. p. Injection for induction of type 1 diabetes. After one week of streptozotocin injection, animals showing glycosuria (fasting blood sugar level>200 mg/dL) were considered as diabetic. The hypoglycemic activity and glucose tolerance test were studied in normal and Streptozotocin-induced diabetic rats after administration of DiaKure at a dose of 300 mg/kg. Blood glucose was determined by a glucose monitor.

Results: At a dose of 200 and 300 mg/kg p. o., DiaKure showed a hypoglycemic effect at a varying degree of significance (P<0.05-0.001) in normal rats in comparison with the respective control group. Maximum effect of DiaKure treatment in the glucose tolerance test occurred at 120thminute of glucose administration in normal rats.

Conclusion: The results indicate significant hypoglycemic activity of DiaKure in male albino rats.

Keywords: Polyherbal, Glucose tolerance test, Dia Kure, Hypoglycemic, Streptozotocin.

INTRODUCTION

Diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The number of people suffering from diabetes has soared to 246 million and the disease now kills more people than AIDS [1]. It is a common disorder among the Indian population. Worldwide incidence of diabetes in the general population is 5%. It is estimated that there are 171 million people in the world with diabetes in the year 2000 and this is likely to increase up to 366 million by 2030[2]. As the number of the people with diabetes multiplies worldwide, the disease has taken an ever-increasing share of national and international healthcare budgets.

In day-to-day practice, most of the diabetic patients were treated with standard anti-diabetic drugs such as sulfonylureas, biguanides, etc. These drugs have some kind of side effects like nausea, vomiting, abdominal pain, diarrhoea, headache, etc., and thus search for a new, safe, and potent anti-diabetic herbal formulation is essential to overcome these problems.

MATERIALS AND METHODS

Herbal medicines are the oldest remedies known to mankind. Herbs had been used by all cultures throughout history, but India has one of the oldest, richest, and most diverse cultural living traditions associated with the use of medicinal plants [3]. Indian plants, which are most effective and commonly studied in relation to diabetes and its associated complications are *Vetiverazizanoidis, Gymnema-sylvestre, Hemidesmusindicus, Azadirachtaindica, Strychnos potatorum, Salacia reticulata, Acasia catechu, Aegle marmelos, Holarhena antidysenterica, Cassia auriculata, Trigonella graecum, Coccinia indica,* and *Syzygium cumini* [4]. The aim of the present study is to develop an anti-diabetic polyherbal formulation without any side effects of the traditional allopathic medications and also reduce the economic burden of the diabetic patients. With the above information in view, "DiaKure", an indigenous polyherbal formulation (containing medicinal plants shown in table 1 below) was developed in RVS College of Pharmaceutical Sciences, Coimbatore. This formulation has been selected to evaluate its anti-diabetic activity in this study.

Table 1: Names of the herbs and their families present in polyherbal formulation (DiaKure)

S. No.	Botanical name	Family name
1	*Vetiveria zizanioides*	Graminaceae
2	*Hemidesmus indicus*	Asclepiadaceae
3	*Strychno spotatrum*	Loganaceae
4	*Salacia reticulata*	Hippocrateacea
5	*Acacia catechu*	Mimosoidaceae
6	*Holarrhena antidysenterica*	Apocyanaceae
7	*Cassia auriculata*	Caesalpiniaceae
8	*Trigonella graecum*	Fabaceae

Animals

Healthy albino rats of either sex of 2-2½-months-old of body weight 125-150 g were housed in polypropylene cages at 25±2°C with light dark cycle of 12 h in the Animal House of RVS College of Pharmaceutical Sciences. It was acclimatized for seven days. All animals were given standard rat feed and water ad libitum [5]. The experiments were performed after approval of the protocol by the

Institutional Animal Ethics Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (Registration No. 1012/c/06/CPCSEA).

Test drug and chemicals

DiaKure is a poly herbal powder formulation, which contains all the herbs as noted in the table 1. All the herbs were collected from an herbal store and botanical garden of RVS Ayurveda Hospital, Coimbatore. Individual drugs were identified and authenticated by comparison with herbarium specimens at Tamil Nadu Agricultural University Coimbatore. All the individual herbs were washed and dried properly in shade. Dried constituents were made into powder in a grinder and sieved properly.

They were then mixed together to make the poly herbal formulation and is stored in an air-tight container free from moisture. Streptozotocin was obtained from the Sigma Chemical Co., St. Louis, MO, USA. All chemicals used in the study were of analytical grade.

Induction of diabetes in experimental animals

Experimental diabetes was induced by single i. p. Injection of 60 mg/kg of streptozotocin (STZ), freshly dissolved in cold citrate buffer (pH 4.5) after 15 min of i. p. injection of nicotinamide (110 mg/kg) prepared in normal saline. Rats with marked glycosuria (fasting blood glucose level greater than 200 mg/dL) after one week of administration of STZ was used for the study [6].

Glucose tolerance test

The Oral Glucose Tolerance test (OGTT) measures the body's ability to use glucose, which is the body's main source of energy. Oral glucose tolerance test was performed in overnight fasted (18 h) normal rats. Normal rats were divided into four groups, each consisting of six rats. Group I was normal control (0.5% of CMC solution). Group II and III animals received different concentrations of DiaKure viz., 200 mg/kg and 300 mg/kg respectively.

Group IV animals are standard receiving Glibenclamide (GL) 10 mg/kg body weight. Groups II and III animals were treated orally with a single dose of DiaKure at a dose of 200 mg/kg and 300 mg/kg p. o. respectively. Doses were fixed after performing proper toxicological evaluations as per OECD guidelines (407,423). Drug was administered using oral gavage method. Glucose (2 g/kg) was fed orally through orogastric tubes 30 min after the administration of the drug [7]. Control animals were administered with equal volume of water. Blood was withdrawn from the tail vein at 0, 1, 2, 3 and 4 hr of glucose administration and blood glucose levels were determined by the glucose oxidase method using glucometer (Accuchek active) [8]. Results were tabulated in table 2. The percentage induced glycaemia (%IG) following oral glucose load at different time intervals was calculated for the control and treated groups as follows.

$$\%IG = (Gx\text{-}Go)/Go \times 10$$

Where Go is the initial glycemia (mg/dL) and Gx the glycemia (mg/dL) at different time intervals after the oral glucose load [9].

Hypoglycemic activity

On the basis of the OGTT studies in normal and diabetic rats, dose was selected for STZ-induced diabetic rat model.

Experimental design

All diabetic rats were randomly divided into four groups of six rats in each groups, 24rats (18 diabetic rats and 6 normal rats).

Group I–Normal control (0.5% w/v CMC sol.)

Group II–Diabetic control (0.5% w/v CMC sol.)

Group III–Streptozotocin+Glibenclamide (10 mg/kg p. o)

Group IV–Streptozotocin+300 mg/kg body weight of DiaKure.

The test drug DiaKure was suspended in a vehicle containing 0.5% w/v CMC in distilled water and administered orally using an oro-gastric tube once daily for 20 d. The body weight of the animals was measured at the onset of the study and at the regular intervals of every week up to 28 d [10].

Group I animals were administered orally with distilled water whereas group II animals received streptozotocin, group III received glibenclamide (10 mg/kg p. o) [11] and group IV received DiaKure 300 mg/kg body weight for 28 consecutive days.

The animal dose has been derived from the human dose by using standard dose ratios. The blood samples collected from the tail vein of rats on 0, 7, 14, 21 and 28 d after administration of formulation. The blood glucose levels were determined by the glucose oxidase method using glucometer (Accucheck active)[8]. Results were tabulated in table 3.

Statistical analysis

All values are expressed as mean±SEM. Statistical analysis was performed by One-way Anova, analysis of variance (ANOVA) followed by Dunnet's t-test. A 'p' value less than 0.05 was considered significant.

Effect of dia kure on glucose-loaded rat (OGTT Model)

Vehicle treated group and GL (10 mg/kg body wt) treated group showed 43.4% and 9.0% rise in serum glucose level (SGL) after one hour of glucose administration where as groups II and III showed 20.5% increase and 21% increase in SGL respectively. From the study, it was found out that both 200 mg/kg and 300 mg/kg of DiaKure possess significant hypoglycemic activity in normal rats. It is found that 200 mg/kg of DiaKure showed a 13% reduction in blood glucose at second hour and 300 mg/kg of DiaKure shows 16.5 % reduction at the same time interval compared to 11.3% decrease and 3.4% decrease in control group and GL group respectively, shown in table 2.

Hence, DiaKure 300 mg/kg dose were selected for further study in STZ-induced diabetic rat model. However, all groups of animals almost normalized the SGLs within three hours indicating that the pancreas of animals was healthy to clear out the glucose load from the body.

Table 2: Effect of DiaKure on serum glucose levels in OGTT model in normal rats

S. No.	Drug/Control	Body weight	Blood glucose level (mg/dL)				
			0 hour	1 hour	2 hour	3 hour	4 hour
1	Group-1 control (distilled water)	180.0±2.0	92.0±2.5	132.0±3.5	117.0±0	119.0±1.0	100.5±1.5
2	Group-2 DiaKure (200 mg/kg)	164.1±2.7	102.0±1.0 **	123±0 **	107.0±2.0 **	101.0±3.0 *	98.0±2.0 *
3	Group-3 DiaKure (300 mg/kg)	152.6±3.4	99.0±1.5 **	120.0±1.5 **	100.0±2.5 **	96.0±3.0 *	88.5±1.5 *
4	Group-4 GL (10 mg/kg body wt)	151.3±2.3	111.0±4.5 **	121.0±3.1 **	117.0±3.6 **	114.0±2.6 *	112.5±1.2 *

Values are represented as mean±SEM (n=6 rats), Values are statistically significant at *P<0.05,** P<0.01, GL = Glibenclamide.

Table 3: Effect of 27 d treatment of Dia Kure on serum glucose levels of STZ-induced diabetic rats

S. No.	Treatment	Initial	7th day	14th day	21stday	28th day
1	Normal control	89.3±3.8	91.0±1.5	95.0±1.0	92.8±2.1	89.0±1.7
2	Diabetic control	221.5±3.2	267.3±3.5	310.3±2.2	383.0±2.8	405.3±3.2
3	Diabetic+Glibenclamide (10 mg/kg)	281.0±1.9 ***	261.0±3.6 **	153±3.8 ***	140.1±3.4 ***	129.5±2.7 ***
4	Diabetic+Diakure (300 mg/kg)	240.1±2.2 ***	210.6±3.3 ***	160.3±3.7 ***	121.3±1.4 ***	96.8±1.7 ***

Values are represented as mean±SEM (n=6 rats), Values are statistically significant at ** P<0.01, *** P<0.001. Diabetic+DiaKure compared with diabetic+glibenclamide and normal control rats.

Effect of Dia Kure on serum glucose level of diabetic rats

Diabetic control rats showed the consistent and gradual rise in SGL during the study. GL (10 mg/kg body wt) and Dia Kure 300 mg/kg treated rats showed a reduction in SGL by 7.1%, 45.5%, 50.1, 53.9%; and 12.3%, 33.3%, 49.5%, 59.7% on 7th, 14th, 21st, and 28th day of the study and the results were found to be statistically significant (P<001) as compared to diabetic control which is shown in table 3. The effect was found to be time dependent up to 28th day of the study. Decrease in SGL was more significant (P<0.001) on 28th day when compared with the standard drug.

There was also a significant reduction in body weight in diabetic animals, however, the animals treated with 300 mg of DiaKure and GL showed significant (P<0.001) check on the loss of body weight on days 21 and 28 in comparison to the day of onset of the study. This effect may be attributed to increased insulin secretion and food consumption. This is shown in table 4. These results implied that the developed polyherbal formulation DiaKure can reduce the complications of body weight and associated cardiovascular risk factors during diabetes.

Toxicity study

Throughout the study, the animals treated with the developed polyherbal formulation DiaKure did not show any behavioral changes and mortality, however, a detailed liver function test should be conducted to ascertain the risk.

The poly herbal formulation DiaKure is a mixture of some potent anti-diabetic herbal drugs. For performing an OGTT, the normal rats were treated with 2 mg/kg of oral glucose solution after an overnight fasting. Normal rats were used for checking the efficacy of DiaKure. Oral ingestion of glucose solution results in an elevated blood sugar level. The elevated blood sugar can be reduced with DiaKure administration. During OGTT, the blood samples were collected at a time interval of 0,1,2,3 & 4 h. The glycemic level of DiaKure treated groups at different doses are compared with control groups. The experiment showed that the body's ability to maintain the glycemic control may be measured by OGTT in normal albino rats. Anti-hyperglycemic effect of DiaKure was checked in Streptozotocin-induced diabetic albino rats after an 18 h fasting. Glibenclamide 10 mg/kg is used as a standard. The diabetic rats were subjected for 28 d study ad libitum.

Table 4: Effect of DiaKure treatment on body weight in STZ-induced diabetic rats on 21st day and 28th day

S. No.	Drug/Control	Body weight(g)		
		Baseline	21st day	28th day
1	Normal control	180.0±2.0	180.9±3.2	182.2±3.1
2	Diabetic control	164.1±7.1	155.0±7.0	123±10.2 **
3	Diabetic+Glibenclamide (10 mg/kg)	152.6±8.4	153.8±9.5 **	155.1±6.7 ***
4	Diabetic+DiaKure (300 mg/kg)	151.3±7.3	152.0±5.1 **	156.0±7.3 ***

Values are represented as mean±SEM (n=6 rats), Values are statistically significant at ** P<0.01, *** P<0.001. Diabetic+DiaKure compared with diabetic+glibenclamide and normal control rats.

Streptozotocin is probably the most widely used agents producing insulin-dependent diabetes mellitus and non-insulin dependent diabetes mellitus in experimental animals. Streptozotocin is a glucosamine nitrosourea compound [12]. STZ causes beta cells of islets of Langerhans of rats to clearly degenerate. In three days, Streptozotocin makes pancreas swell and at last causes degeneration in beta cells of islets of Langerhans and induces experimental diabetes. It also changes normal metabolism in diabetic rats in comparison with normal rats. Consumption of water and food, the volume of urine, serum glucose increases in diabetic animals in comparison with normal rats, but the levels of serum insulin, C-peptide and body weight decreases [13]. The characteristic loss of body weight is due to increased muscle wasting in diabetes [14]. When diabetic rats were treated with DiaKure, the weight loss was put on the check and reversed.

It is found that DiaKure has significant hypoglycemic effect in normoglycemic rats and anti-hyperglycemic effect in streptozotocin-induced diabetic rats. Expected activity of the drug is compared with that of existing marketed antidiabetic drug.

From the study, it is concluded that the crude polyherbal formulation possess significant anti-hyperglycemic activity, which can be used effectively to control the elevated blood sugar in diabetics. Further studies need to be carried out to find out the active principles of the drug and mechanism of action.

CONFLICT OF INTERESTS

All authors have none to declare.

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